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ALTERATIONS IN PLASMA AMINO ACID HOMEOSTASIS DURING EXPERIMENTA--ETC(U)
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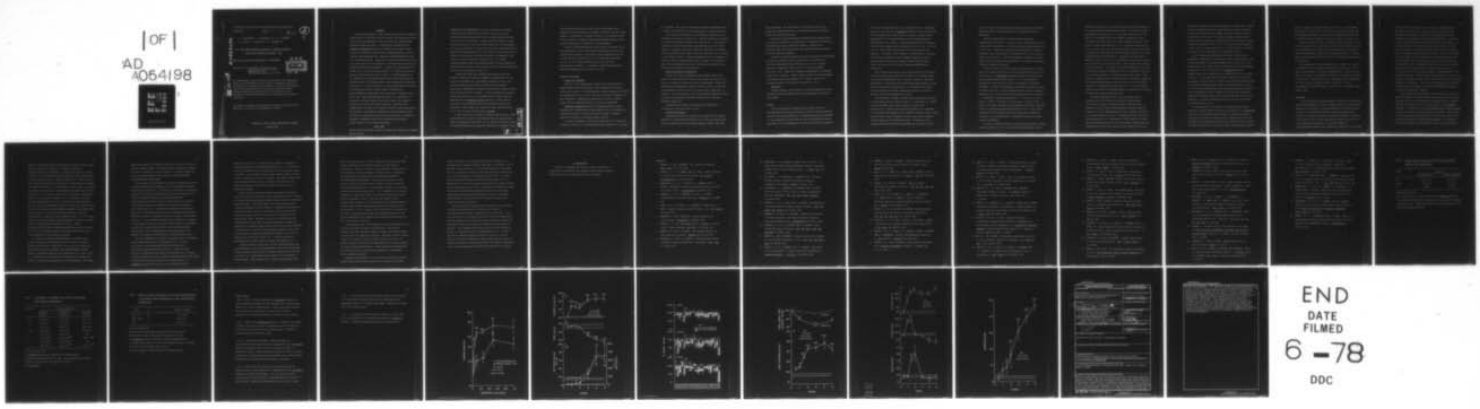

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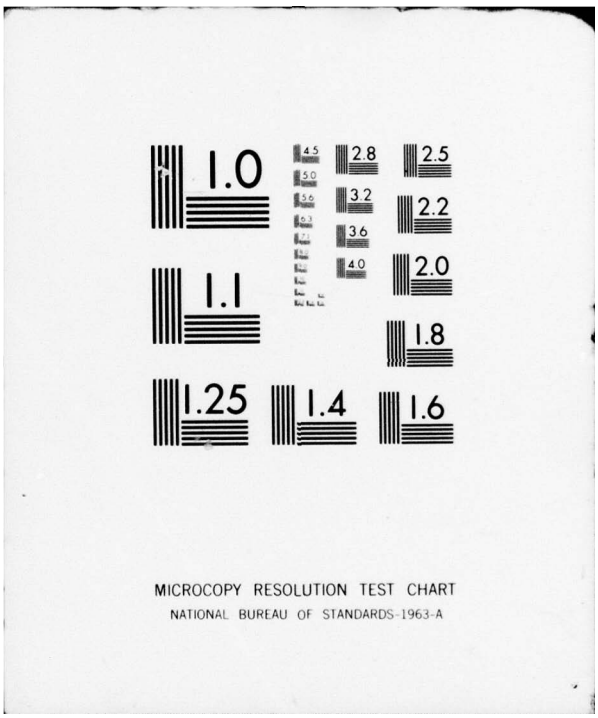
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Alterations in plasma amino acid homeostasis during experimental endotoxemia

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Running head: AMINO ACID HOMEOSTASIS IN ENDOTOXEMIA

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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on the Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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ABSTRACT

Studies were performed in rats to determine the effect of endotoxin administration on individual plasma free amino acid concentrations since altered amino acid homeostasis could be involved in pathophysiologic mechansims during endotoxemia. Intraperitoneal administration of Salmonella typhimurium endotoxin (1.0 mg/100 g body weight) induces an early and persistent hypertaurinemia, hyperglucagonemia, as well as transient hyperinsulinemia and a generalized hypoaminoacidemia during a 5-h experimental period. Depressions in plasma concentrations of most amino acids appear to be attributable to endotoxin-induced elevations in peripheral glucagon and ureagenesis. Arginine nearly disappeared from plasma within 3 h and was not detectable by 5 h. Marked alteration in arginine concentration may be due, in part, to hyperglucagonemia and the liberation of intracellular arginase from various tissues and its enhanced activity in peripheral blood. Overt hepatocellular damage was detectable by 3 h and may contribute to altered plasma glucose and amino acid homeostasis during the latter stages of endotoxicosis. Hypertaurinemia was demonstrable with a wide range of endotoxin doses (0.05-1 mg). Early hypertaurinemia may result as a consequence of destruction of formed elements of the blood, whereas the sustained condition may involve more complex mechanisms. Decreased renal clearance does not appear to be involved in altered taurine homeostasis. The physiologic and metabolic consequences of hypertaurinemia during endotoxemia are unknown but may involve certain aspects of temperature regulation, carbohydrate metabolism, and behavioral alterations commonly observed in rats during endotoxemia.

Index Terms

Taurine; Endotoxin-induced; Glucagon; Insulin; Rats; Plasma zinc; Arginase; Hepatic glycogen

DECREASES IN THE CONCENTRATION of total as well as most individual plasma free amino acids have been reported to occur in man and experimental animals during the course of various bacterial (53) and viral (10, 19, 52) infections. In many instances sequential changes during acute stage of infectious disease occur prior to the appearance of overt clinical signs of illness and fever and are characterized by a generalized hypoaminoacidemia (52, 53). However, the type and extent of change in the free amino acid pattern appears to be dependent on the severity as well as duration of infection (53). For example, hyperaminoacidemia rather than hypoaminoacidemia has been demonstrated during the chronic stage of viral illness (10) and in severe gram-positve (25) as well as gram-negative sepsis (24).

Although endotoxemia may accompany gram-negative sepsis and endotoxin induces both impaired carbohydrate metabolism (9, 33) and hepatocellular damage (45), the possible influence of toxin on the infection-related plasma animo acid changes and the potential physiological significance of sequential concentration changes in amino acids has received little attention. In work performed more than two decades ago, Dooley et al. (17) provided initial evidence that the administration of Salmonella pullorum endotoxin to chicks induces depressions in the concentrations of blood arginine, glycine, and methionine. These changes were identical to those reported by Ross et al. (42) for chicks infected with S. pullorum.

In this study we have extended our previous work with rats, which demonstrated the occurrence of hyperaminoacidemia and hepatocellular damage in experimental endotoxemia (46), to document further and define the endotoxin-induced early sequential alterations in peripheral amino

acid concentrations during the acute stage of endotoxemia and to determine the possible hormonal mechanisms involved in certain of these changes. In particular, we focused attention on our observation (46) of the occurrence of endotoxin-induced hypertaurinemia because of the potential physiological significance of this phenomenon.

Taurine (2-aminoethanesulphonic acid) has been postulated to be a neurotransmitter (2) and to have numerous biological functions which include either regulation or modulation of body temperature (26, 28), certain behavioral patterns (31, 44, 49), carbohydrate metabolism (14, 15, 16, 37), hormone secretion (14, 35, 36), and other physiological processes (2). In addition, taurine has been shown to possess antistaphylococcic activity in vivo (50).

MATERIALS AND METHODS

Animals and Treatments.

Male rats of the Sprague-Dawley (Charles River Laboratories) or Fisher-Dunning (Harlan Laboratories) strain weighing 225-275 g were housed ten per cage in a room maintained at constant temperature (22-24°C) and lighted from 6 a.m. to 6 p.m. Rats were used after a minimum of one week acclimation. Animals were provided water and food ad libitum except food was withdrawn during the 5-h course of experiments.

Salmonella typhimurium endotoxin, (Lipopolysaccharide B, Difco Laboratories) was administered intraperitoneally (ip) as a suspension in physiological saline at the doses specified in Tables and Figures. Control rats received an equivalent volume of saline.

In certain experiments, rats were pretreated daily for 3 days with a single ip injection of 4-deoxypyridoxine (Sigma Chemical Co.) at a dose

of 3.0 mg/100 g body weight as described by Raghavan and Nadkarni (40) to inhibit the formation of taurine through amino acid decarboxylation.

Liver perfusion experiments were performed by a modified in situ technique as previously described (54). Bile was collected by inserting an indwelling polyethylene catheter into the bile duct. The perfusate consisted of the following: packed sheep erythrocytes, 30 ml; bovine albumin, 3 g; heparin, 500 units; penicillin, 3000 units; streptomycin, 3 mg; and sufficient Krebs-Ringer bicarbonate buffer (pH 7.4) to achieve a total volume of 106 ml. Endotoxin suspension (1.0 ml, 10 mg/ml) or an equivalent volume of physiological saline was added to the perfusate after a 60-min equilibration period. Perfusate was recirculated for a 90-min period subsequent to equilibration.

Sample Collection and Preparation.

Animals were lightly anesthetized with halothane; whole blood samples were obtained from the pleural cavity after transsection of the vena cava. Heparin (10 IU/ml) was used as anticoagulant. Plasma was obtained after centrifugation (1500 x g, 15 min at 5°C) of whole blood and subsequently stored at -20°C for no longer than 3 weeks prior to analyses. In some experiments, livers were extirpated immediately after sacrifice and processed for glycogen determination by previously described methods (21).

Plasma samples for amino acid analyses were treated with sulfosalicyclic acid (53) prior to assay.

Analytical Procedures.

Hematological values were obtained with standard Coulter methods (Model ZF-5, Curtin Matheson Scientific, Inc.) with the exception that platelet concentrations were determined manually (Unopette Test 5855,

Becton-Dickenson). The distribution of leukocytes in whole blood samples was determined manually by performing duplicate differential counts from stained blood smears.

Plasma amino acid analyses were performed with an automated amino acid analyzer according to the manufacturer's procedure for operation in the physiological mode (121M-TB-013 Beckman). Quantitation of individual amino acid concentrations was achieved with Beckman System AA on-line computing integrator.

Previously described procedures were used to determine concentrations of plasma Zn, glucagon, insulin, glucose (21), and activities of ornithine carbamoyltransferase (OCT) E.C.2.1.3.3. (45), glutamic oxalacetic transaminase (SGOT) E.C.2.6.1.1. (30), and arginase E.C.3.5.3.1. (41). Plama urea nitrogen was determined by an automated method (N-1C, Technicon). Hepatic glycogen content was also determined by previously described procedures (21). Urinary creatinine was performed according to the method of Chasson et al. (13).

Statistics

Unpaired Student *t* test was used to determine significance of the difference between experimental and control group means at specified time periods.

RESULTS

Initial studies were performed with two strains of rats to determine whether interstrain differences existed in endotoxin-induced lethality and certain parameters. The maximum endotoxin dose employed, 1.0 mg, represented an LD_{70} (N=20) for the Fisher-Dunning rats and an LD_{10} (N=20) for the Sprague-Dawley strain when mortality was assessed at

24 h after toxin administration. Data presented in Fig. 1 demonstrate that the ip injection of S. typhimurium endotoxin in doses ranging from 0.05-1.0 mg/100 g body weight induces an increase in plasma taurine concentration 5 h after administration in both Fisher-Dunning and Sprague-Hawley rats. However, a significant difference exists between these strains in plasma taurine concentrations prior to ($P < 0.01$) and after ($P < 0.005$-0.001) the administration of various amounts of endotoxin. The plasma taurine response to graded amounts of endotoxin in both strains appeared to reach a maximum; however, the dose of endotoxin which illicited this apparent maximum differed between strain, 0.1 mg and 0.5 mg for Fisher-Dunning and Sprague-Dawley, respectively.

Rats of the Fisher-Dunning strain were selected for further study since they appeared more sensitive with respect to the plasma taurine response than Sprague-Dawley rats. Therefore, results of subsequent experiments to be presented were obtained using Fisher-Dunning rats and an arbitrarily selected endotoxin dose of 1.0 mg/100 g body weight. This dose produced an apparent maximum effect but did not result in mortality during the experimental 5-h period.

The time-course of change in plasma taurine concentrations during a 5-h experimental period is shown in Fig. 2A. A significant ($P < 0.001$) increase in plasma taurine occurred within 1 h after toxin administration. Elevated taurine levels persisted for the subsequent 4-h period with some suggestion that a biphasic response existed. Plasma taurine levels in control animals at various time intervals remained constant during the 5-h experimental period. In addition to the hypertaurinemia described above, measurements of the urinary taurine

output over the 24-h period following endotoxin administration (Table 1) provided evidence that hypertaurineuria is a prominent sequela of endotoxemia.

Endotoxin administration is known to depress plasma Zn concentrations in rats (38). A significant ($P < 0.001$) depression in Zn concentration was evident at 3 h with an apparent maximum depression at 5 h (Fig. 2B). Plasma Zn concentrations were measured to confirm the endotoxemic state and because of its postulated role in the release of taurine from tissue (1). Under the experimental conditions, elevated plasma taurine concentrations occurred both in the absence and presence significant plasma Zn depression.

Since the liver is a critical organ in the maintenance of plasma amino acid concentrations (43) and we have previously reported (45, 46) that the dose of S. typhimurium endotoxin employed (1.0 mg/100 g body weight) induces hepatocellular damage at 5 h, it was pertinent to determine the time-course and extent of such damage in the present experiments. Results of measurements of plasma OCT and SGOT activities are shown in Fig. 2C. In close agreement with our previous findings, a significant ($P < 0.01$) elevation of both enzyme activities occurred at 4 h. Progressively increasing values were noted as early as 3 h with respect to SGOT ($P < 0.05$) and 1.5 h with respect to OCT ($P < 0.05$). In general, the plasma acitivities of both enzymes increased in a parallel manner with apparent maximum values obtained at 4-5 h. These results indicated that no significant detectable hepatocellular damage was present at 1 h, the earliest time of significant elevation in plasma taurine concentration.

In order to determine whether the observed early increase in plasma taurine occurred as part of a generalized hyperaminoacidemia, other

individual plasma amino acid concentrations were determined during the 5-h period following endotoxin administration. The results of these measurements are shown in Fig. 3. The plasma concentrations of the majority of individual amino acids measured, expressed as percent of initial (zero time) values, were significantly depressed ($P < 0.05$) within 1 h with an apparent nadir at 3 h. Comparable changes did not occur in control rats. The relative depression in plasma concentration of arginine was most marked and decreased rapidly during the 3-h period following endotoxin administration. Arginine was not detectable in endotoxemic rats at 5 h. By 5 h, considerable variation was observed in the concentration of many amino acids in control animals when compared to zero time values. In contrast, the marked depression in most amino acids readily apparent in endotoxemic rats at 3 h appeared to abate by 5 h with some suggestion of a reversal toward initial values. When changes in plasma taurine concentrations are not considered, significant ($P < 0.005$) hypoaminoacidemia occurred as early as 1 h and persisted through the 5-h experimental period (Fig. 4A). At the time periods studied, the presence of hypoaminoacidemia was accompanied by increased concentrations of urea nitrogen (Fig 4B).

The results presented in Fig. 5A provide evidence that a significant ($P < 0.05$) increase in peripheral plasma glucagon concentration occurred 1 h following endotoxin administration. A significant ($P < 0.01$-0.001) hyperglucagonemia persisted during the remainder of the 5-h experimental period with a biphasic time-course; with apparent maxima at 2 and 5 h. Concomitant with the increase in plasma glucagon at 1 h, a significant ($P < 0.01$) elevation in plasma glucose concentration (Fig. 5B) occurred; however, plasma glucose was significantly ($P < 0.05$) decreased at 5 h when values for both time

periods were compared to glucose concentrations in control rats. Hepatic glycogen in endotoxemic rats was significantly ($P < 0.001$) decreased at 5 h (0.27 $\pm$ 0.03 mg/g wet liver weight, N=10) when compared to control values (18.60 $\pm$ 2.20, n=10). The initial increase in plasma glucose concentration was followed by a significant ($P < 0.001$) transient increase in insulin concentration (Fig. 5C) at 1.5 h with an apparent maximum at 2 h and a return to basal levels at 3 h. No further increase in insulin was observed for the remainder of the experimental period.

Several studies were performed in an attempt to elucidate the mechanism(s) responsible for the endotoxin-induced hypertaurinemia; they included measurements of peripheral blood counts; taurine output from isolated livers, and plasma taurine concentrations after deoxypyridoxine pretreatment of rats. The effect of S. typhimurium endotoxin administration on the concentrations of peripheral blood cells is shown in Table 2. It is evident that the amount of endotoxin (1.0 mg/100 g body weight) previously shown to produce an early hypertaurinemia induced a significant leukocytopenia ($P < 0.001$) and thrombocytopenia ($P < 0.01$) 1.5 h after toxin administration which persisted through the remainder of the 5-h experimental period. Although not statistically significant, leukocyte and platelet counts began to decrease as early as 0.5 h after endotoxin. A persistent erythrocytopenia ($P < 0.001$) was also evident but occurred later, at 3 h.

In marked contrast to the effect of systemically administered endotoxin on plasma taurine concentration, the addition of S. typhimurium endotoxin to recirculating perfusate fluids (0.1 mg/ml) of isolated livers did not increase the taurine output in these fluids (2.62 $\pm$ 0.20 µmol/100 g body weight, mean $\pm$ SE, N=5) when compared to those obtained for saline controls (2.65 $\pm$ 0.36, N=5) during a 90 min perfusion period.

However, consistent with the observation of Utili et al. (51) addition of endotoxin did significantly ($P < 0.05$) decrease bile flow during the 90 min perfusion period; 2.75 $\pm$ 0.09 µl/g body weight (mean $\pm$ SE, N=5) for endotoxin-treated compared to 3.13 $\pm$ 0.14 (N=5) for control livers.

The data presented in Table 3 demonstrate that pretreatment of rats with deoxypyridoxine in an attempt to prevent taurine formation via amino acid decarboxylase (40) failed to prevent the occurrence of endotoxin-induced hypertaurinemia during the 5-h postendotoxin period. Treatment with deoxypyridoxine alone also failed to alter endogenous plasma taurine concentrations.

The disappearance of plasma arginine by 5 h prompted us to perform an additional study to determine plasma arginase activity for various time periods subsequent to endotoxin administration. Arginase has been shown to be capable of promoting the conversion of arginine to ornithine in plasma (12). Results shown in Fig. 6 demonstrate that a significant ($P < 0.01$) increase in enzymic activity occurred at 1.5 h with approximately a ten-fold increase in activity at 5 h as compared to the control values.

DISCUSSION

We have previously reported preliminary findings (46) that endotoxin induced hyperaminoacidemia in rats concomitant with overt hepatocellular damage which was evident 5 h after administration of a lethal quantity of toxin. In the present experiments, hyperaminoacidemia did not develop; we attribute the apparent contradictory findings to variability in the time of onset of severe hepatocellular damage and in potency of various endotoxin preparations. The possible findings of hyperaminoacidemia together with hepatocellular damage is not surprising

in view of the key role which the liver plays in regulating plasma free amino acid levels (43). However, additional factors such as decreased renal clearance and amino acid utilization may possibly contribute to the previously observed hyperaminoacidemia. Our results do not allow discrimination between these and other possibilities. It is likely, however, that extensive hepatocellular damage is a major contributing factor to elevated plasma free amino acid concentrations. Recently, hyperaminoacidemia has been reported to occur in the presence of hepatic damage in mice inoculated with mouse hepatitis virus (10), in rats injected with the hepatotoxin, CCl_4 (12), and in humans with viral hepatitis (19). The apparent inability of the liver to regulate amino acid levels during the latter stages of endotoxemia may be associated in part with the known impairment in gluconeogenesis (9, 33) induced by endotoxin. Extensive hepatocellular damage, at least in rats, appears to be casually related to both altered amino acid and carbohydrate metabolism in endotoxemia. To the best of our knowledge, few metabolic studies involving endotoxemia have included longitudinal measurements to evaluate hepatocellular integrity.

In the present study, evidence has been presented that indicates, with the exception of increased plasma taurine concentration, that most individual amino acid concentrations are decreased within a few hours following the administration of a lethal quantity of S. typhimurium endotoxin. In addition, the early hypoaminoacidemia and hypertaurinemia occurred in the absence of any detectable liver damage. The depression in most plasma amino acids observed in the present study appears to be attributable primarily to marked hyperglucagonemia. The finding of increased plasma urea nitrogen during the course of the experimental

period is consistent with this interpretation because of the well known effect of glucagon on ureagenesis. Although the effect of hyperinsulinemia on the depression of plasma acids cannot be excluded, Griffin et al. (23) have proposed that insulin has only a minimal effect on plasma amino acid levels. However, Pozefsky et al. (39) have shown that insulin-stimulated amino acid uptake by skeletal muscle can depress the plasma concentration of a limited number of amino acids. The ability of glucagon to reduce plasma concentrations of most amino acids is well known (8, 19, 32). However, plasma taurine and cysteine concentrations appear to be uneffected by glucagon (19). The finding of endotoxin-induced hyperglucagonemia in rats is consistent with that observed in man (48), dogs (4), and subhuman primates (5) after toxin administration. The early and persistent hyperglucagonemia observed in the present study evidently becomes less effective in depressing plasma amino acid concentrations if significant liver pathology develops. It is not known whether the ovserved sustained hyperglucagonemia is related to continued secretion, impaired hepatic degradation, or both. Initial hypoaminoacidemia followed by hyperaminoacidemia has also been reported to occur in mice during the course of infection with hepatitis virus (10) as significant liver pathology developed.

Our finding of transient hyperinsulinemia is consistent with a previous observation in endotoxemic rats (9). Others, in studies involving infected (24) or endotoxemic dogs (4) could not demonstrate hyperinsulinism, with the exception that hyperinsulinemia could be induced in endotoxemic dogs after the administration of a glucose load (4). Blackard et al. (4) reported that endotoxin did not induce the release of insulin from rat pancreatic islet preparations. This latter

observation suggests that endotoxin does not act directly on β-cells to stimulate insulin release. No similar evidence is available concerning the effect of endotoxin on α-cell secretion. Our results suggest that in the rat hyperinsulinemia occurs secondary to glucagon-induced glycogenolysis and hyperglycemia.

The physiological implications and potential mechansism(s) involved in hyperinsulinemia during endotoxicosis have recently been discussed by Buchanan and Filkens (9). In addition, our observation of concomitant elevations in glucagon and insulin might possibly be explained by the release of endogenous mediators from endotoxin-stimulated polymorphonuclear leukocytes, which act either directly or indirectly on the endocrine pancreas to stimulate the simultaneous release of insulin and glucagon (21). Working with crude mediator preparations, George et al. (21) demonstrated in rats that ip administration induced a significant elevation in peripheral insulin and glucagon concentrations within 1 h. If these leukocytic mediators are involved in our findings, it would suggest that they are produced, perhaps released, and initiate the pancreatic response within 30 min or less following endotoxin administration, since hyperinsulinemia and hyperglucagonemia were apparent in the present study within 90 min following administration.

The near disappearance of arginine from plasma within 3 h following endotoxin administration and its disappearance by 5 h could be attributable to both hyperglucagonemia and the liberation of intracellular arginase induced by endotoxin. Glucagon is highly effective in depressing plasma arginine concentrations (8). Elevation in plasma arginase activity has previously been demonstrated in man during typhoid fever and in rats following the ip administration of S. typhimurium endotoxin (29) as well as in rats injected with the

hepatotoxin, CCl_4 (11). The conversion of arginine to ornithine in plasma by arginase has also been demonstrated (11, 12). In addition to liver (11), leukocytes (6), and erythrocytes (47) are potential sources of arginase. In our studies, plasma arginine content rapidly decreased without a concomitant increase in ornithine. This finding suggests that plasma arginase activity, may not contribute significantly to the observed disappearance of arginine, however, we cannot exclude its possible involvement.

Perhaps the most provocative finding with respect to endotoxin-induced alterations in plasma amino acid concentrations is the occurrence of an early and persistent hypertaurinemia during a period of generalized hypoaminoacidemia. The general shape of the curve relating plasma taurine concentration to various amounts of ip administered endotoxin is compatible with the concept that liberation of this amino acid from taurine-rich tissue sites is, at least in part, responsible for the observed high levels. Although our results do not allow definitive identification of these sites, it appears reasonable to suggest that destruction of peripheral leukocytes and platelets may provide sufficient released taurine to explain the hypertaurinemia especially since these cells maintain extremely high concentration gradients, with a cell to plasma ratio in the order of 500:1 (47). A role for platelets in modulating plasma taurine levels has recently been suggested (7).

Evidence obtained in this study, with the isolated perfused liver and pretreatment of rats with deoxypyridoxine, indicated that endotoxin-induced increased hepatic release or enhanced formation of taurine does not appear to be a contributing factor involved in the mechanisms of hypertaurinemia. Since skeletal muscle contains approximately 75% of

the total taurine content of the rat, liberation from this tissue could possibly contribute to the observed increase in plasma taurine concentration. However, to the best of our knowledge, endotoxin has not been reported to cause early damage to skeletal muscle. Furthermore, the administration of plasmocid (8-3-diethylaminopropylamino-6-methoxyquinoline) dihydroiodide, a muscle-necrotizing agent in rats, failed to reduce the taurine content of muscle in spite of plasmocid-induced hypertaurineuria (22). The mechanisms involved in the continued hypertaurinemia occurring during the 3- to 5-h interval in our study may undoubtedly be complex and consist of continued release from the blood cellular compartment as well as from liver and lymphoid tissue. Decreased renal clearance as a contributing factor in hypertaurinemia is unlikely since increased urinary taurine output was found after toxin administration. It is of interest to note that this 3- to 5-h period is the time of pronounced hypozincemia; some relationship between Zn and taurine metabolism has been suggested and increased taurine release may occur in Zn-deficient states (1).

An additional factor to be considered in the taurine response is the potential involvement of adrenal corticosteroids. Destruction of lymphoid tissue with subsequent release of taurine from this particularly rich site (3) may be promoted by endotoxin. Endotoxin is known to induce a rapid early increase in adrenal corticol output in rats (34). In addition, Dubreuil and Timeras (18) demonstrated that cortisone treatment in rabbits induced hypertaurinemia. To what extent these considerations are actually involved in altered taurine metabolism of endotoxemia, however, remains speculative.

Although results obtained in this study with different rat strains suggest that altered taurine metabolism is not related to the lethal

aspects of endotoxin, the potential involvement of taurine in the pathophysiologic responses induced by endotoxins is intriguing. This is especially true with respect to its reported induction of hypothermia (26, 28), potentiation of insulin action (16) and glucose oxidation (15, 16), as well as depression of food and water drive (50). It is well known that endotoxin-treated rats demonstrate hypothermia (20, 27), hypoglycemia (9), as well as sedation and depression in eating, drinking, and bar-pressing work activity (27). Recently in our laboratory, Bailey and co-workers (unpublished communication) found that ip endotoxin administration prevents mouse-killing behavior in rats. Peripheral administration of taurine has also been reported to decrease this particular behavior in rats (31).

Initial studies with endotoxemic rats conducted in our laboratory confirmed the hypothermia which occurred in rats after the intracerebroventricular (ICV) administration of taurine (44). In an interesting series of experiments, Harris and Lipton (26) demonstrated that ICV administration of taurine in rabbits effectively prevented the hyperthermia induced in this species by peripheral administration of endotoxin. They suggested that taurine may interact with endogenous pyrogen in a manner which alters its biological activity. In view of the numerous proposed biological functions of taurine, in systems known to be altered by endotoxin, it seems apparent that more work is required concerning the potential physiologic and metabolic consequences of the elevated plasma taurine concentrations reported in this study.

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TABLE 1. Urinary taurine excretion for the 24-h period following endotoxin (LPS) administration

Group	N	Taurine	
		(μmol/100 g/24 h)	(μmol/mg creatinine)
LPS	6	34.6 ± 2.2	22.9 ± 1.6
Saline	6	6.8 ± 0.9	10.2 ± 1.2
P		<0.001	<0.001

Values are means ± SE.

Rats received either 1 mg/100 g body weight S. typhimurium endotoxin or an equivalent volume of physiological saline at the time of placement in metabolic cages. Animals were allowed water ad libitum but not food during the experimental period.

TABLE 2. Concentration of peripheral blood cells at various times after endotoxin administration

Time (h)	Cells (No./mm^3) (N)		
	Leukocytes ($x10^3$)(5)	Erythrocytes ($x10^6$)(5)	Platelets ($x10^3$)(3)
0	10.3 ± 0.5	7.24 ± 0.12	801 ± 52
0.5	9.9 ± 0.5	7.41 ± 0.31	727 ± 43
1	9.3 ± 1.5	6.94 ± 0.37	581 ± 119
1.5	6.3 ± 0.5***	7.29 ± 0.32	422 ± 50*
2	6.2 ± 0.7**	7.26 ± 0.30	ND
3	4.6 ± 0.6***	5.21 ± 0.27***	158 ± 27***
4	3.8 ± 0.6***	5.00 ± 0.46**	ND
5	3.4 ± 0.4***	5.34 ± 0.32***	111 ± 1***

Values are means ± SE.

S. typhimurium endotoxin was administered intraperitoneally (1.0 mg/100 g body weight) at zero time. Values significantly different from zero time; *P <0.01, **P <0.005, ***P <0.001.

†Not determined.

TABLE 3. Effect of multiple pretreatment of rats with 4-deoxypyridoxine on the plasma taurine concentration 5 h after endotoxin (LPS) administration

Group	N	Taurine (μmol/dl)
DPN + LPS	10	71.5 ± 8.8*
DPN + Saline	10	22.0 ± 0.9
None	5	22.4 ± 1.7

Values are means ± SE.

Rats received a single daily ip injection of DPN dissolved in physiological saline (2.0 mg/100 g body weight) for 3 days. S. typhimurium endotoxin suspended in saline was administered ip 1.0 mg/100 g body weight) 22 h after the last DPN injection. The effect of LPS alone was previously shown in Fig. 1.

*P <0.001 compared to saline-treated or no-treatment groups.

FIGURE LEGENDS

FIG. 1. Effect of various amounts of S. typhimurium endotoxin on plasma taurine concentrations in Fisher-Dunning (FD) or Sprague-Dawley (SD) rats 5 h after ip administration. Values significantly different from controls of the same strain are noted with an asterisk. Significance for interstrain differences is noted in Results Section.

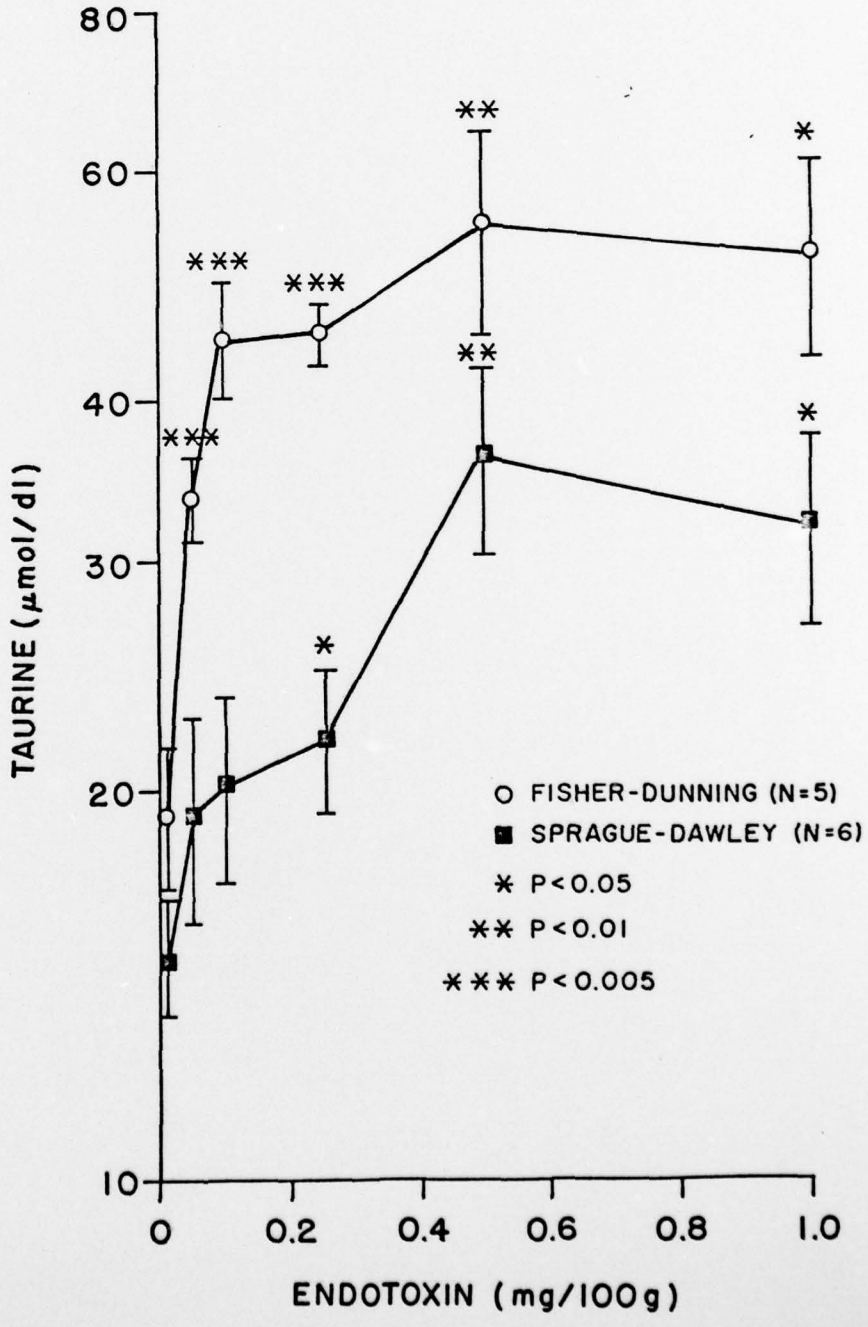
FIG. 2. Effect of S. typhimurium endotoxin, 1.0 mg/100 g body weight, on plasma taurine (A), and Zn (B) concentrations and OCT and SGOT (C) activities at various times after ip admininstration. Shaded area represents mean ± SE for control values.

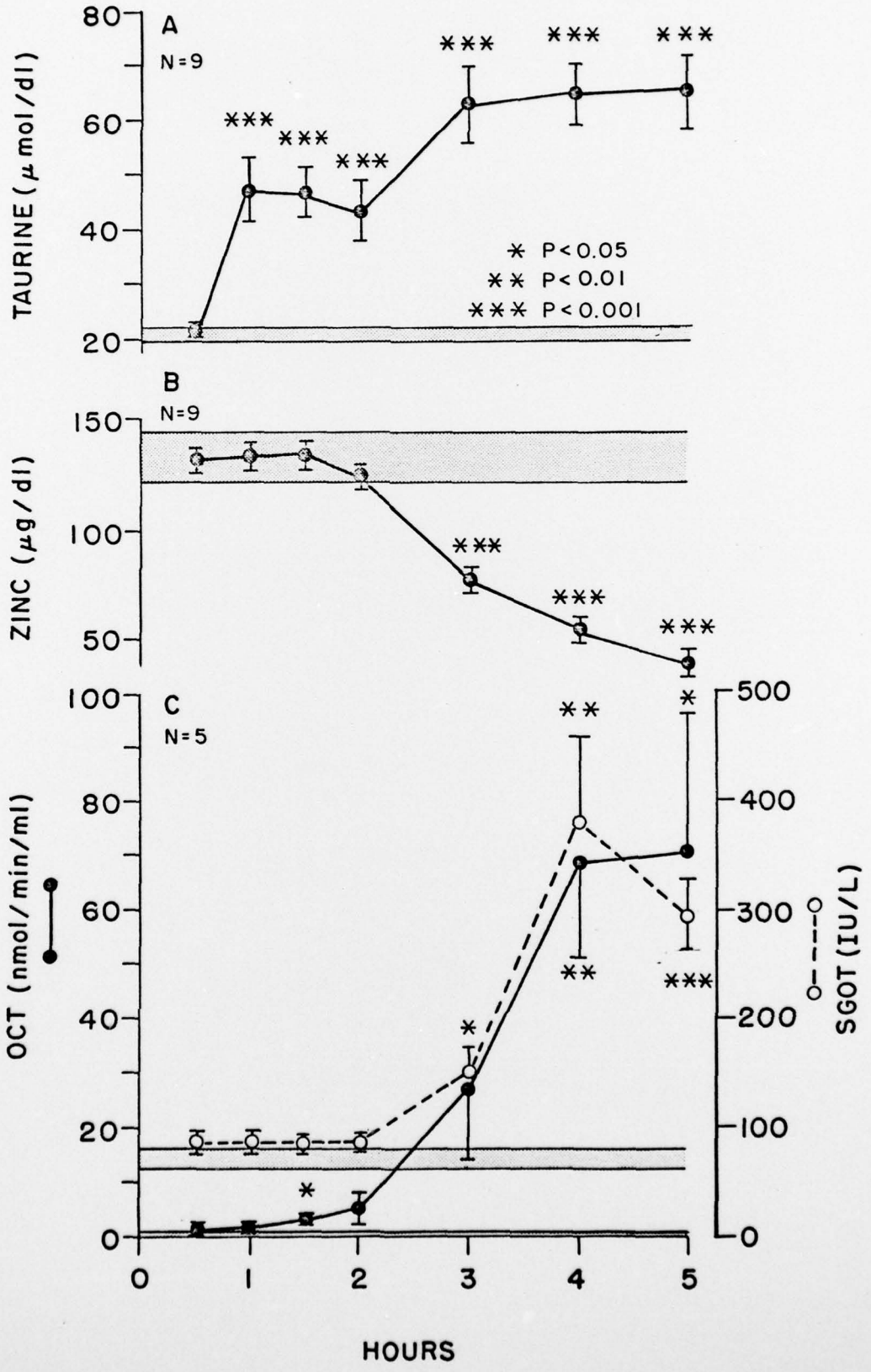
FIG. 3. Alterations in individual plasma free amino acid concentrations at various times after ip administration of S. typhimurium endotoxin (1.0 mg/100 g body weight) or an equivalent volume of physiological saline. Values expressed as percent of initial (zero time) concentration. Asterisk above an below individual amino acids indicates significance for control and endotoxin groups, respectively.

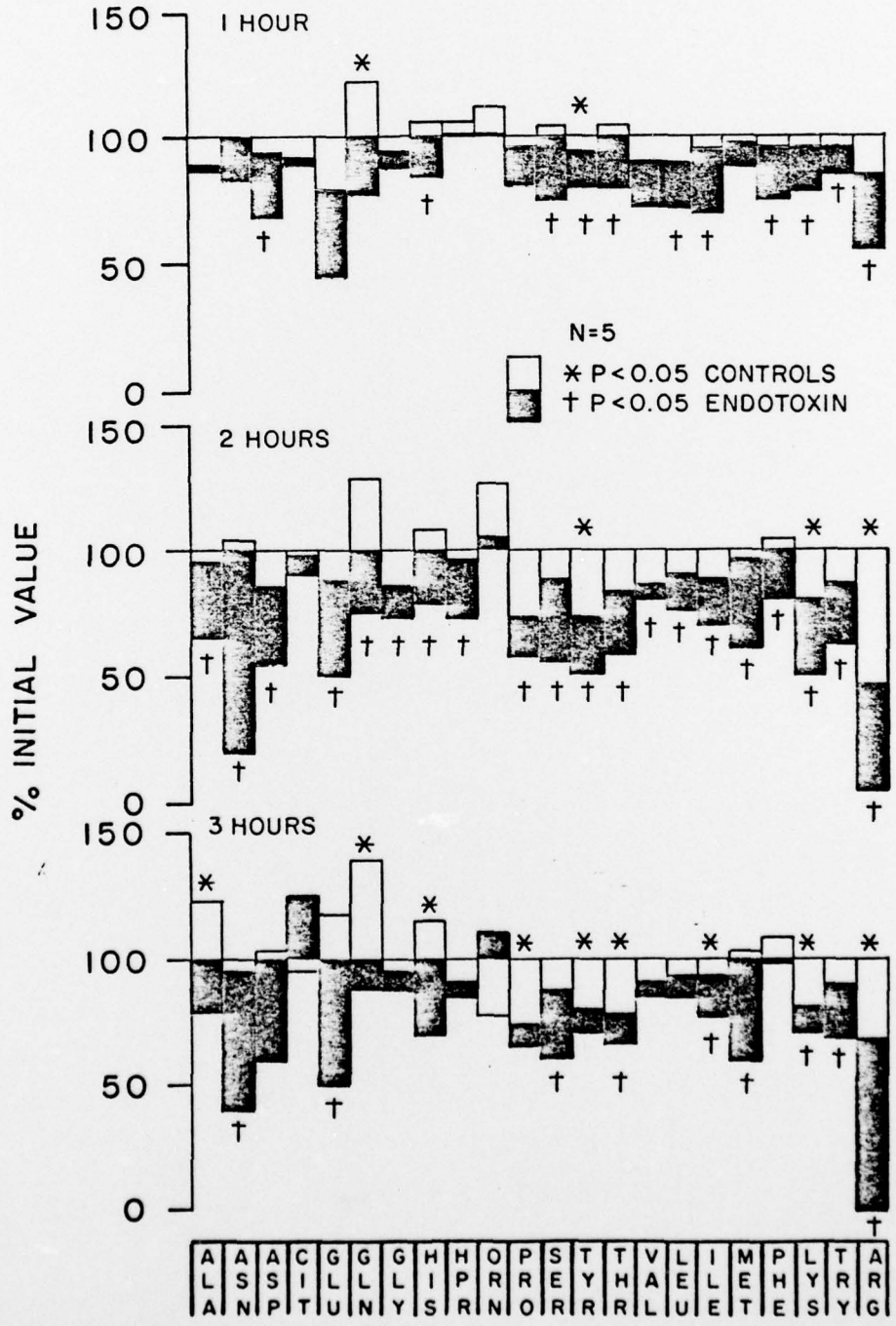
FIG. 4. Plasma total free amino acid (A) and urea nitrogen (B) concentrations at various times after ip administration of S. typhimurium endotoxin (1.0 mg/100 g body weight). The total amino acid value represents the mean and includes all individual amino acids (see Fig. 3) except taurine. Shaded area represents mean ± SE for control values.

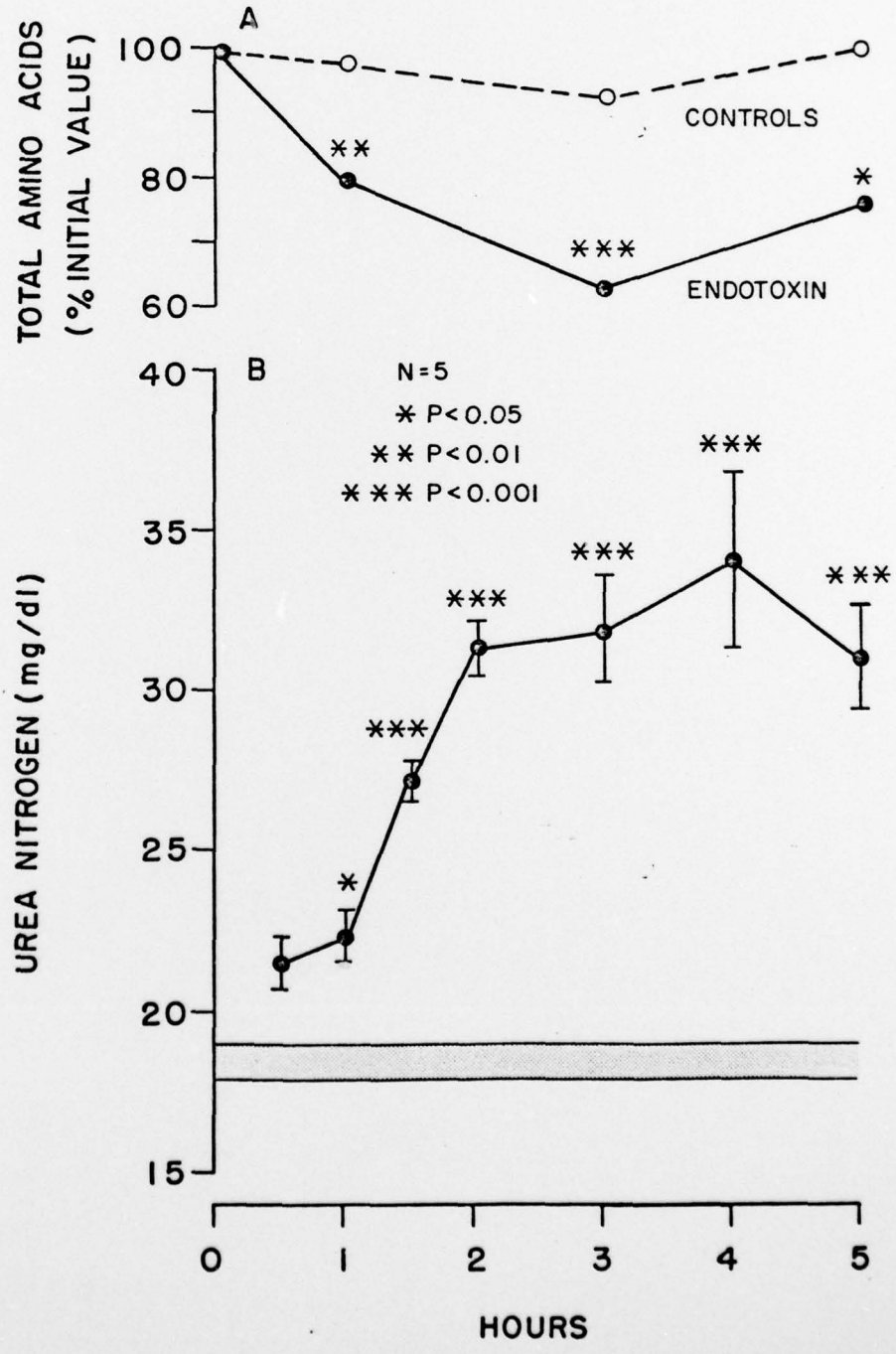
FIG. 5. Alterations in plasma glucagon (A), glucose (B) and insulin (C) concentrations at various times after ip administration of S. typhimurium endotoxin, 1.0 mg/100 g body weight. Shaded area represents mean ± SE of control values.

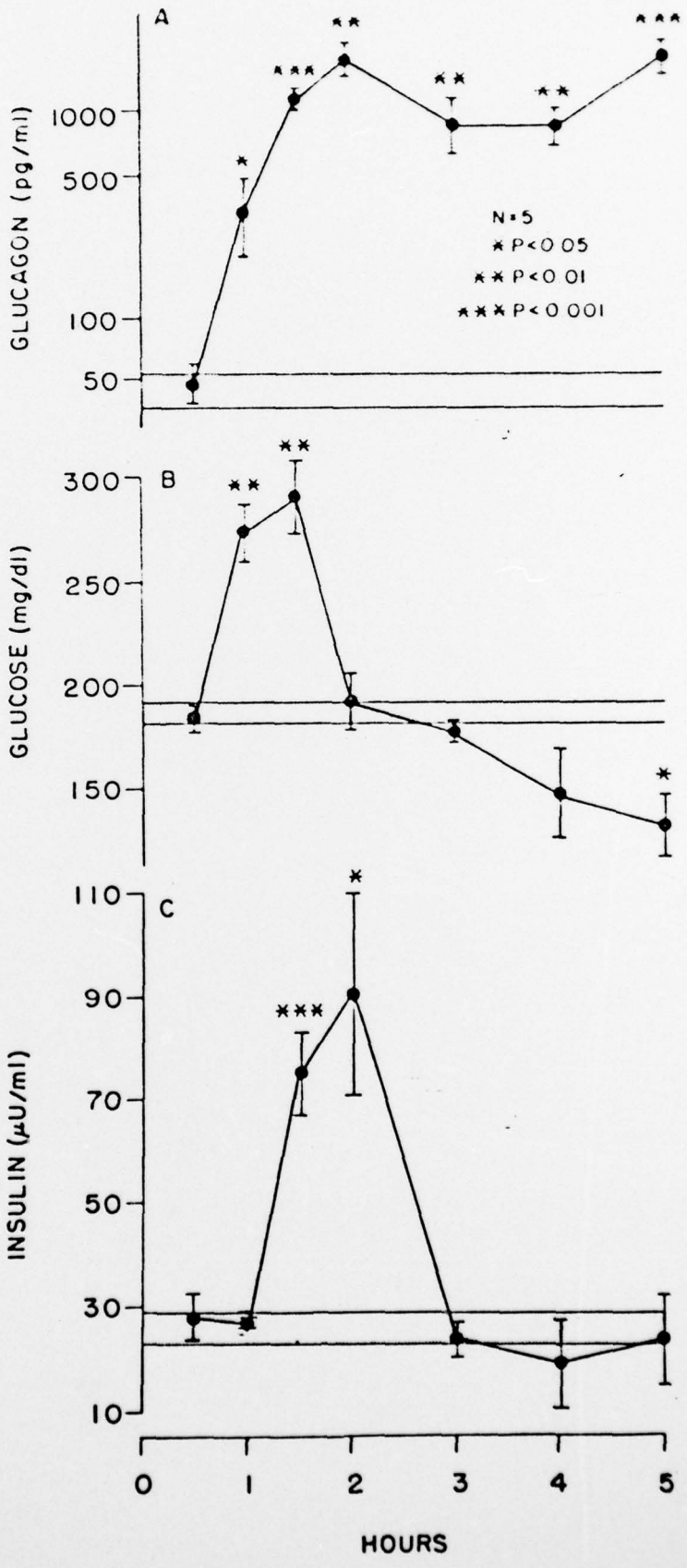
FIG. 6. Alterations in plasma arginase activity at various times after ip administration of 1.0 mg/100 g body weight S. typhimurium endotoxin. Shaded area represents mean ± SE of control values.

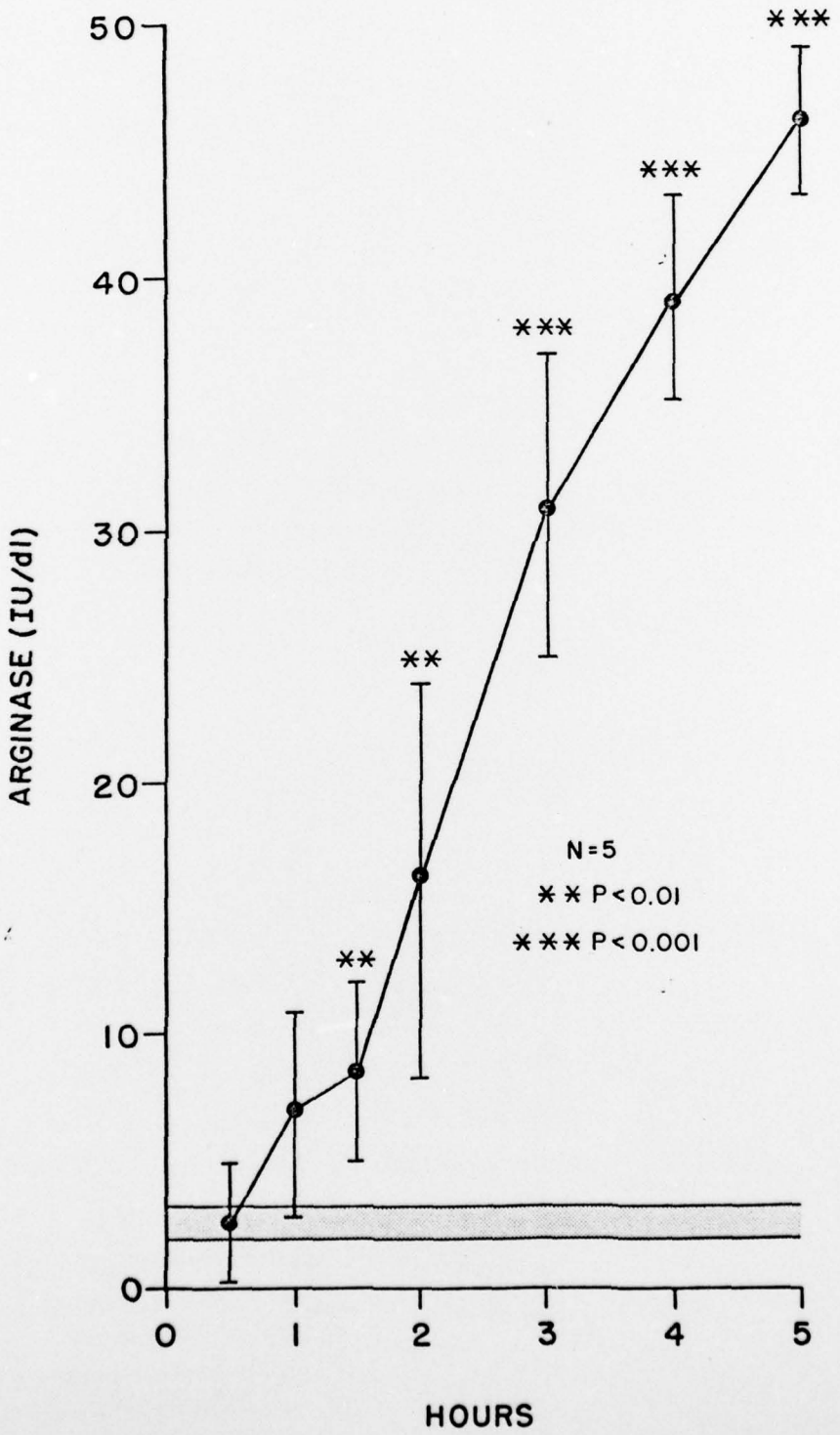

80
60
40
30
20
10
TAURINE (μmol/dl)
0
0.2
0.4
0.6
0.8
1.0
ENDOTOXIN (mg/100g)
FISHER-DUNNING (N=5)
SPRAGUE-DAWLEY (N=6)
* P<0.05
** P<0.01
*** P<0.005


A
N=9
TAURINE (μ mol/dl)
80
60
40
20
* P<0.05
** P<0.01
*** P<0.001
B
N=9
ZINC (μg/dl)
150
100
50
C
N=5
OCT (nmol/min/ml)
SGOT (IU/L)
100
80
60
40
20
0
500
400
300
200
100
0
0
1
2
3
4
5
HOURS


150
100
50
0
1 HOUR
2 HOURS
3 HOURS
N=5
* P<0.05 CONTROLS
† P<0.05 ENDOTOXIN
% INITIAL VALUE
ALA
ASN
ASP
CIT
GLU
GLN
GLY
HIS
HPR
ORN
PRO
SER
TYR
THR
VAL
LEU
ILE
MET
PHE
LYS
TRY
ARG


A
TOTAL AMINO ACIDS
(% INITIAL VALUE)
100
80
60
CONTROLS
ENDOTOXIN
**
***
*
B
N=5
* P<0.05
** P<0.01
*** P<0.001
UREA NITROGEN (mg/dl)
40
35
30
25
20
15
0
1
2
3
4
5
HOURS


A
GLUCAGON (pg/ml)
1000
500
100
50
N=5
* P<0.05
** P<0.01
*** P<0.001
B
GLUCOSE (mg/dl)
300
250
200
150
C
INSULIN (μU/ml)
110
90
70
50
30
10
0
1
2
3
4
5
HOURS


50
40
30
20
10
0
ARGINASE (IU/dl)
0
1
2
3
4
5
HOURS
***
***
***
**
**
N=5
** P<0.01
*** P<0.001

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Studies were performed in rats to determine the effect of endotoxin administration on individual plams free amino acid concentrations since altered amino acid homeostasis could be involved in pathophysiologic mechanisms during endotoxemia. Intraperitoneal administration of Salmonella typhimurium endotoxin (1.0 mg/100 g body weight) induces an early and persistent hypertaurinemia, hyperglucagonemia as well as transient hyperinsulinemia and a generalized hypoaminoacidemia during a 5-h experimental period. Depressions in plasma concentrations of most amino acids appear to be attributable to endotoxin-induced elevations in peripheral

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glucagon and ureagenesis. Arginine nearly disappeared from plasma within 3 h and was not detectable by 5 h. Marked alteration in arginine concentration may be due, in part, to hyperglucagonemia and the liberation of intracellular arginase from various tissues and its enhanced activity in peripheral blood. Overt hepatocellular damage was detectable by 3 h and may contribute to altered plasma glucose and amino acid homeostasis during the latter stages of endotoxicosis. Hypertaurinemia was demonstrable with a wide range of endotoxin doses (0.05-1 mg). Early hypertaurinemia may result as a consequence of destruction of formed elements of the blood, whereas the sustained condition may involve more complex mechanisms. Decreased renal clearance does not appear to be involved in altered taurine homeostasis. The physiologic and metabolic consequences of hypertaurinemia during endotoxemia are unknown but may involve certain aspects of temperature regulation, carbohydrate metabolism, and behavioral alterations commonly observed in rats during endotoxemia.